

Evidence of Biogenic Corrosion of Titanium After Exposure to a Continuous Culture of Thiobacillus Ferrooxidans Grown in Thiosulfate Medium

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**EVIDENCE OF BIOGENIC CORROSION OF TITANIUM AFTER EXPOSURE TO A
CONTINUOUS CULTURE OF *Thiobacillus ferrooxidans*
GROWN IN THIOSULFATE MEDIUM**

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ABSTRACT

Experiments were undertaken to evaluate extreme conditions under which candidate materials intended for use in a proposed nuclear waste repository might be susceptible to corrosion by endogenous microorganisms. *Thiobacillus ferrooxidans*, a sulfur-oxidizing bacterium, was grown in continuous culture using thiosulfate as an energy source; thiosulfate is oxidized to sulfate as a metabolic endproduct by this organism. Culture conditions were optimized to produce a high-density, metabolically active culture throughout a period of long term incubation in the presence of Alloy 22 (a high nickel-based alloy) and Titanium grade 7 (Tigr7) material coupons. After seven months incubation under these conditions, material coupons were withdrawn and analyzed by high resolution microscopy and energy dispersive x-ray analyses. Alloy 22 coupons showed no detectable signs of corrosion. Tigr7, however, demonstrated distinct roughening of the coupon surface, and [presumably solubilized and precipitated] titanium was detected on Alloy 22 coupons incubated in the same *T. ferrooxidans* culture vessel. Control coupons of these materials incubated in sterile thiosulfate medium did not demonstrate any signs of corrosion, thus showing that observed corrosive effects were due to the *T. ferrooxidans* metabolic activities. *T. ferrooxidans* intermediates of thiosulfate oxidation or sulfate may have caused the corrosive effects observed on Tigr7.

INTRODUCTION

The United States Department of Energy has been mandated to design and construct a repository for the storage of high level commercial and defense-related nuclear waste. The current design of the repository is centered on a geologic, subterranean concept coupled with an engineered barrier system

(EBS) composed of corrosion resistant waste packages covered by a drip shield. Presently, the waste package design includes a corrosion resistant outer barrier of Alloy 22 (UNS N06022, a nickel-based alloy) overlaying a structural support of Stainless Steel 316L (S31603). The candidate material composing the drip is Titanium. Microorganisms resident in the geologic horizon in which the proposed repository will be emplaced, and those introduced as a result of construction and operational activities may contribute to corrosion of these materials, thereby compromising containment of nuclear waste. Therefore, efforts are under way to determine both the potential modes of Microbiologically Influenced Corrosion (MIC), as well as quantify these effects, and evaluate the conditions under which they might occur.

Titanium and Alloy 22 may be susceptible to attack by select reduced sulfur compounds and sulfuric acid, although nickel-based alloys have been reported more immune to the effects of sulfuric acid. Reduced forms of sulfur are generally more damaging than those that are oxidized, and thiosulfate has been reported to cause pitting¹. Titanium is known to be resistant to attack by a wide range of chemical compounds, including some reduced sulfur species and organic acids². Additionally, titanium has been reported to be largely immune to MIC, although it can become fouled by colonization with microorganisms³. Nickel-based alloys are less characterized with respect to their resistance to MIC. Our previously reported studies regarding the susceptibility of Alloy 22, however, showed corrosive effects of a community of subsurface bacteria; corrosion rates were estimated to be on the order of $1.2\mu\text{m}/\text{yr}$ ⁴. Electrochemical experiments demonstrated that these bacteria were increasing corrosion rates of Alloy 22 by a factor of at least two when compared to abiotic corrosion under identical conditions⁵. Little and co-workers reported etching of a stainless steel containing 6% molybdenum but no effects on titanium (grade 2) in the presence of *Thiobacillus ferrooxidans* grown in ferrous iron medium under batch conditions using electrochemical techniques⁶.

The present work was undertaken to evaluate extreme conditions under which these materials might be susceptible to MIC. *T. ferrooxidans*, a sulfur- and iron-oxidizing bacterium, were grown in continuous culture, with a constant supply of a growth medium containing thiosulfate as an energy source, generating sulfuric acid as an end product of metabolism. Culture conditions were aimed at generating an exponential-phase culture in which the organisms were metabolically vigorous over an extended period; material coupons were exposed to this continually growing culture throughout the incubation period of seven months. Our findings show that there was no discernable corrosion of Alloy 22, however Titanium grade 7 (UNS R52400) did show signs of generalized corrosion on a micrometer scale using atomic force microscopy (AFM). Consistent with signs of Titanium corrosion observed using AFM, deposition of [presumably soubilized] Titanium was found on Alloy 22 coupons resident in the same bioreactor containing *T. ferrooxidans* and Titanium coupons. Titanium coupons exposed to sterile thiosulfate medium did not display any signs of corrosion, thereby implicating *T. ferrooxidans* metabolic products of thiosulfate as potential corrosive agents on Titanium.

EXPERIMENTAL METHODS

Organism and Culture Conditions

Thiobacillus ferrooxidans #21834 was obtained from the American Type Culture Collection (ATCC) and grown in *Thiobacillus ferrooxidans* Medium with Thiosulfate⁷ (Thiosulfate Medium). Thiosulfate Medium contained (per liter) 5.0 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 3.0 g KH_2PO_4 , 3.0 g $(\text{NH}_4)_2\text{SO}_4$, 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; pH was adjusted to 4.4 before sterilization. Stationary phase pre-cultures were grown at 26°C with agitation before being inoculated (1:50) into a sterilized culture vessel composed of a modified 2.5 L stirred cell (Cytostir, Kontes) containing sterilized metal coupons

in teflon trays and 2 L. of Thiosulfate Medium (Fig. 1); both Alloy 22 and Titanium coupons were resident in the same stirred cell reactor. Stirred cells were incubated at 26° C. on a stir plate to enable aeration via the vessel's magnetically-driven paddle mechanism. Growing cultures were continuously supplied (3 mL/hr.) with Thiosulfate Medium through a vessel inlet attached to a two 1 L. medium reservoirs. A peristaltic pump transferred fresh medium from the reservoir to the stirred cell, and spent media and cells were removed (3 mL/hr., also using a peristaltic pump) into a waste receptacle through a vessel outlet. Outlet tubing included two media breaktubes in series to prevent back-contamination from the waste receptacle.

Material Specimens

Alloy 22 (a Ni-Cr-Mo alloy) and Titanium grade7 (Tigr7) material coupons (3.23 cm² surface area per coupon) with machined surfaces (32RMS) were stamped with an identifying number, and cleaned with distilled water and acetone, before being weighed for eventual gravimetric analysis. Coupons were then sterilized using a ⁶⁰Co gamma radiation source (3-4 Mrad total dose), and placed in teflon trays fabricated to fit into stirred cells. Fifteen coupons of each material were placed in the stirred cell (48.45 cm²/stirred cell/material) using aseptic techniques to maintain sterility; coupons were physically separated from each other by the teflon dividers comprising the tray (Fig. 1). To effectively assess the microbiological contributions to observed results, sterile control coupons (prepared identically to those emplaced in stirred cells) were placed in flasks containing 200 mL of sterile Thiosulfate Medium; sterile control Alloy 22 and Titanium coupons were incubated in separate flasks.

Preparative and Analytical Techniques

Bulk Solution. Initially and periodically, samples of bulk aqueous solution effluxed from stirred cells were collected. Samples of effluxed solution were split into separate aliquots, one of which was assayed for cell density using a particle counter counter (Coulter-Beckman, Multisizer II, 30 µm aperture). A second aliquot was subjected to analysis of pH.

Material Coupons. Alloy 22 and Tigr7 coupons (Table 1) were removed from the stirred cell containing growing *T. ferrooxidans* and the sterile control vessel after incubation for seven months using sterile techniques. Immediately upon removal, coupons were fixed using the hexamethyldisilazane method (Nation, 1983; Vandivivere, 1992) to image biofilm, scale, and corrosion products using scanning electron microscopy (SEM). Following initial SEM analyses, coupons were washed in 2M HCl for one minute (ASTM method C.6.1) to remove biofilms, scales, and corrosion products and re-analyzed by SEM and Energy Dispersive Spectral analysis (EDS). To gain greater resolution in topological analysis HCl-treated coupons were then exposed to Atomic Force Microscopic (AFM) evaluation. The results of these analyses were all compared to those of coupons which had not been exposed to either stirred cell or sterile control conditions, but which had been subjected to all preparative techniques (i.e., fixation and acid washing).

SEM/EDS. Material coupon surfaces were characterized with a field emission scanning electron microscope (Hitachi S-800) equipped with a Kevex 8000 microanalysis system for image and elemental analyses, respectively. The effective electron beam probe depth was 1 µm. Operating parameters for imaging and energy dispersive spectral analysis (EDS) were 16 kV accelerating voltage, 0-10.23 keV range, approximately 1500 cts/sec, and <20% deadtime. The beam was cropped or operated in spot mode during elemental analysis.

AFM Analysis. Atomic Force Microscope (AFM) images were collected with a Digital Instruments Dimension 3100B head operated in the tapping mode.

RESULTS

Thiobacillus ferrooxidans can utilize both reduced iron and sulfur compounds as energy/electron sources, in the case of thiosulfate, converting $S_2O_3^{2-}$ (S^{+2}) to sulfuric acid, H_2SO_4 (S^{+6}) in a step-wise fashion. Both thiosulfate and sulfuric acid have corrosive properties. These organisms are additionally capable of fixing atmospheric or dissolved inorganic carbon (carbon dioxide or carbonate) to supply cellular carbon, thus their nutritional needs do not include reduced organic carbon sources, such as carbohydrates.

T. ferrooxidans -like organisms have been isolated from a number of samples of rock within the proposed repository geologic horizon, thus these organisms will likely occur within the repository⁸. Since their nutritional needs are minimal, once water becomes available, growth is highly likely if an energy source is also present. In an effort to determine the impacts of *T. ferrooxidans* on the integrity of corrosion resistant materials proposed to compose the EBS, a reference strain of *T. ferrooxidans* was cultured under conditions that support vigorous exponential-phase growth in a potentially corrosive thiosulfate media with the concomitant biogenic production of sulfuric acid. Coupons of candidate EBS materials, Alloy 22 and Titanium, were incubated within the *T. ferrooxidans* stirred cell reactor. Culture conditions were aimed at producing a "worse-case scenario" with respect to possible biocorrosion of proposed EBS materials that might be caused by *T. ferrooxidans*-like organisms in the presence of reduced sulfur compounds. This analysis, therefore, is intended to determine environmental conditions which might promote the biogenic corrosion of these materials, and whether these materials can be corroded under auspicious conditions for both growth and corrosion. Results of the analyses of *T. ferrooxidans*-incubated coupons were compared to unexposed metal coupons as well as coupons incubated in sterile media to separately determine the effects of both media and *T. ferrooxidans* on candidate EBS materials.

Stirred Cell Growth of T. ferrooxidans

Cell reactor influx and efflux rates were targeted to maintain a high-density exponential growth (log-phase) culture (despite washout of cells in effluxing media) for the duration of the incubation period. These conditions enable high rates of sulfuric acid production, while also maintaining a steady state concentration of thiosulfate (from continual feed of media) to evaluate the effects of *T. ferrooxidans* thiosulfate metabolism on material corrosion. Three days following inoculation of the stirred cell reactor, *T. ferrooxidans* concentrations rose to 2.5×10^6 cells/mL, indicating exponential phase growth of the culture. After a number of months incubation, steady state cell concentrations were on the order of 5.8×10^5 cells/mL, again within the bounds of log phase.

To further evaluate growth of *T. ferrooxidans*, reactor efflux was plated on ISP agar plates⁹, which contain reduced ferrous iron (Fe^{+2}) as an energy source. The presence of *T. ferrooxidans* in effluxing reactor medium was readily indicated by growth of "rust-encrusted" colonies on ISP plates caused by oxidation of ferrous iron contained in the plate medium to the ferric state with subsequent spontaneous oxidation to ferric oxides. Further, while the initial pH of the reactor medium was adjusted to 4.4, the pH decreased after growth of the culture to as low as pH 3.1 due to the biological production of sulfuric acid, the endproduct of thiosulfate oxidation. Maintenance of a relatively high cell density within the reactor coupled with the direct observation of thiobacilli growth on indicator plates, and a decrease in pH of the effluxing medium, all served to demonstrate the continued growth and activity of the *T. ferrooxidans* culture within the bioreactor.

Surfacial Imaging and EDS Analysis of Stirred Cell-Incubated Materials

Alloy 22. SEM micrographs of Alloy 22 coupons incubated for seven months in *T. ferrooxidans*-inoculated stirred cells did not show any discernable evidence of corrosion to acid-washed surfaces, when compared to unexposed or media-incubated sterile control coupons (Fig. 2). Fixed sterile control coupons showed some evidence of scale, which also persisted somewhat even after acid washing. EDS analysis of these scale-like regions showed high levels of chloride, a media component, indicative of the precipitation of salts on the metal surface; chloride was observed distributed in all interrogated regions of the sterile-exposed and *T. ferrooxidans*-exposed coupons.

More interesting, was the appearance on both fixed and acid-washed coupons of dark contrast, mottled regions (resulting from the presence of low molecular weight compounds) on both sterile- and *T. ferrooxidans*-incubated coupons; the mottling occurred with greater frequency on sterile control coupons (Fig. 2). EDS spectra of these dark mottled regions on a sterile control, acid-washed coupon showed only the base metal components, presumably if the mottling was caused by a deposition it was too thin and the beam too penetrating to discern its composition. The presence of calcium (another media component) was observed in some mottled regions, but not all.

Spots observed across the coupon that appeared dark on SEM examination were found to be composed of aluminum. While both calcium and chloride are media components, aluminum is not and the origin of this component is not presently known, although it could be a contaminant or result as an artifact due to machining.

Importantly, titanium particles were also discerned by EDS on Alloy 22 coupons exposed to *T. ferrooxidans*. The titanium apparently originated from the dissolution of Tigr7 coupons resident in the same reactor as Alloy 22 coupons; titanium deposits were not found on sterile controls (these were incubated separately from Alloy 22, see Experimental Methods).

AFM analysis of these same unexposed, sterile media-incubated, and *T. ferrooxidans* stirred cell-incubated coupons also demonstrated no significant effects of *T. ferrooxidans* metabolism on the overall integrity Alloy 22 surfaces (after acid washing, Fig. 3). Average RMS (Root Mean Square) factors, an index of the degree of roughness of the surface, were 254 (n=3) for an unexposed Alloy 22 coupon, 189 (n=2) for a sterile media-exposed coupon, and 172 (n=6) for a *T. ferrooxidans*-incubated coupon (Table 2); thus no significant effect of *T. ferrooxidans* was observed on this material under the present experimental conditions. Interestingly, however, when a mottled region of a sterile control coupon was AFM-imaged, it appeared to be somewhat rougher than unmottled regions (Fig. 3), which was supported by an increased average RMS index of 367 (n=1).

In sum, the presence of *T. ferrooxidans*-generated sulfuric acid and thiosulfate medium did not apparently cause any corrosion of Alloy 22 surfaces after seven months of incubation in continuous culture. This finding is in agreement with the known resistance of nickel-based alloys to sulfuric acid-induced damage¹⁰. The thiosulfate media, however, may have caused deposits on the metal surface, generating regions containing compounds of low molecular weight resulting in a mottled appearance in SEM micrographs which appeared rougher with AFM analysis. The fact that mottling appeared less severe on *T. ferrooxidans*-incubated coupons than on sterile controls may indicate that this organism mitigated the effects of the thiosulfate medium on Alloy 22, possibly by lowering the concentration of thiosulfate through consumption and conversion to sulfuric acid.

Titanium grade 7. Fixed but un-cleaned coupons showed salt precipitates on both sterile control and *T. ferrooxidans*-exposed coupons which apparently originated from precipitation of media components. Again, as was the case for Alloy 22, mottled regions appeared on both sterile control and *T.*

ferrooxidans-exposed coupons, and these regions persisted even after acid washing. These regions require further characterization using alternate methods, perhaps Auger or Raman spectroscopy. EDS examination of an acid-washed *T. ferrooxidans*-exposed coupon also again showed the presence of aluminum.

SEM micrographs of Tigr7 incubated for seven months in continuous *T. ferrooxidans* culture and then acid-washed showed a much rougher surface than either unexposed Tigr7 or Tigr7 that had been incubated in sterile Thiosulfate Medium (Fig. 4). Some evidence of corrosion of *T. ferrooxidans*-exposed coupons was demonstrated by what appears to be a general eroding of the surface; this effect was not observed in SEM images of either sterile media control coupons or those that had not been exposed to any experimental protocol (except fixation and acid cleaning).

AFM imaging of Tigr7 coupons confirmed that areas of acid-washed *T. ferrooxidans*-exposed coupons were indeed somewhat rougher than either sterile control or unexposed acid-washed coupons (Fig. 5). This was reflected in gathered average RMS indices (Table 2), which showed an average RMS of 361 (n=3) for unexposed Tigr7, 409 for a sterile control coupon (n=3), and 519 (n=6) for a *T. ferrooxidans*-incubated coupon.

Taken together, it appears that exposure of Tigr7 for seven months in continuous *T. ferrooxidans* culture resulted in a roughening of the coupon surface that appeared to be indicative of some degree of generalized corrosion. Further evidence of corrosion was provided by the observation of Ti particles on Alloy 22 coupons present in the same reactor. Mottled regions again appeared in SEM images of both media-exposed and *T. ferrooxidans*-exposed coupons; however this appears to be a function of Thiosulfate Medium exposure rather than exposure to *T. ferrooxidans*, since these regions appear in sterile media controls.

DISCUSSION

The effects of exposure of a high nickel-based alloy and titanium to the sulfur-oxidizing bacterium *T. ferrooxidans* using thiosulfate as an energy source (ultimately generating sulfuric acid) were assessed in continuous culture to establish conditions under which these materials might be susceptible to MIC. Alloy 22 was not affected after seven months exposure under these conditions. Grade 7 Titanium, however, did appear to experience attack due to *T. ferrooxidans* metabolism as evidenced by microscopic examination and the finding of [presumably solubilized and] precipitated titanium found on Alloy 22 coupons resident in the same bioreactor. This is the first report, to our knowledge, of possible MIC of titanium. Little and co-workers did not find corrosion of titanium grade 2 under batch culture conditions of *T. ferrooxidans* using ferrous iron as an energy source⁶. These investigators observations may have resulted due to the exhaustion of nutrients in batch culture, resulting in cells becoming less metabolically active, or because the experiments were of shorter duration. However, perhaps the most important factor to explain the difference in findings between this study and those of Little, were the respective choices of growth substrate. The former study used ferrous iron which is oxidized to ferric iron by *T. ferrooxidans*, and had little apparent effect on titanium. In subsequent experiments, these same workers demonstrated no corrosion of titanium after exposure to the thermophilic sulfur-oxidizing organism, *Sulfolobus acidocaldarius*, using a growth medium apparently containing reduced carbon as an energy source¹¹. Again the choice of growth substrate was almost certainly key to the findings; organotrophic metabolism of reduced carbon by *S. acidocaldarius* produces carbon dioxide¹², which is not damaging to titanium. Here, in contrast, the oxidation of thiosulfate by *T. ferrooxidans* appeared to affect some degree of generalized corrosion after long term exposure. In contrast, thiosulfate alone, as shown by sterile controls, demonstrated little corrosion on tested titanium coupons.

The highest oxidation state of sulfur (S^{+6}), contained in sulfuric acid, has generally been known to be less corrosive than more reduced forms of sulfur¹³. As shown above, it appears that thiosulfate (S^{+2}) has no discernable corrosive effect on titanium. Thus, it might be possible that some generation of reduced sulfur, such as elemental sulfur (S^0) or sulfite/sulfurous acid (SO_3^{-2}/H_2SO_3 ; S^{+4}) occurred either biotically or abiotically in the system described here and affected the observed corrosion of titanium. Alternatively, sulfuric acid generated as a final endproduct of metabolism may have affected the observed results. The extreme resistance of titanium to corrosion has been attributed to the stability of an overlying titanium oxide passive film¹¹. Therefore, if corrosion of titanium is affected by any of these sulfur-containing compounds, then it most probably is attacking the passive film.

The biotic production of sulfur and sulfite by *Thiobacillus* from thiosulfate has been well characterized (Fig. 6). Elemental sulfur generation occurs due to splitting thiosulfate into sulfite and sulfur by this organism, sulfite is then oxidized to sulfate (producing cellular energy), while the sulfur can be oxidized at a later time if the supply of thiosulfate becomes limiting¹⁴. Under the growth conditions used here, thiosulfate is always available, thus generating conditions conducive to the production of sulfur. It may be the elemental sulfur that is affecting corrosion of titanium, or perhaps some sulfite is escaping cells and entering the surrounding growth medium before it is oxidized to sulfate. Further experiments to evaluate the observed results will include determining the type and concentration of sulfur-containing compounds in cultures that could be affecting the observed results. Also, titanium will be incubated in abiotic solutions of the sulfur species found in *Thiobacillus* cultures. Finally, this coupons of both Alloy 22 and Tigr7 and continuing to be incubated in the continuous culture of *T. ferrooxidans* to assess the results reported here.

The results of these experiments and those planned to further characterize these results should allow the elucidation of conditions, both biotic and abiotic, that cause corrosion of titanium. Mechanism(s) of titanium corrosion may have implications for its potential use in the long-term storage of nuclear waste by defining boundary conditions that promote its dissolution. Defining the mechanisms of titanium corrosion may further facilitate better usage of this material for other applications, including those involving chemical processing, oil recovery and refining, water desalinization, and medical implants.

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Table 1. Composition of Alloy 22 and Titanium grade 7 (weight %)

Material	C	Mn	S	Co	Mo	Si	Cr	W	V	Fe	P	Ni	Ti	Pd	N
Alloy 22	.002	0.26	.001	0.51	13.4	0.25	21.6	2.82	0.15	3.95	.012	Bal.			
Ti gr7	0.1									0.3			Bal.	0.25	0.03

Table 2. Averaged RMS Indices of AFM-Interrogated Material Coupons*

Material	Unexposed	Sterile/Media- Only Exposed	<i>T. ferrooxidans</i>- Exposed
Alloy 22	254	189	172
Ti gr7	361	409	519

*RMS data were all collected on 100 μm \times 100 μm area of coupon surface

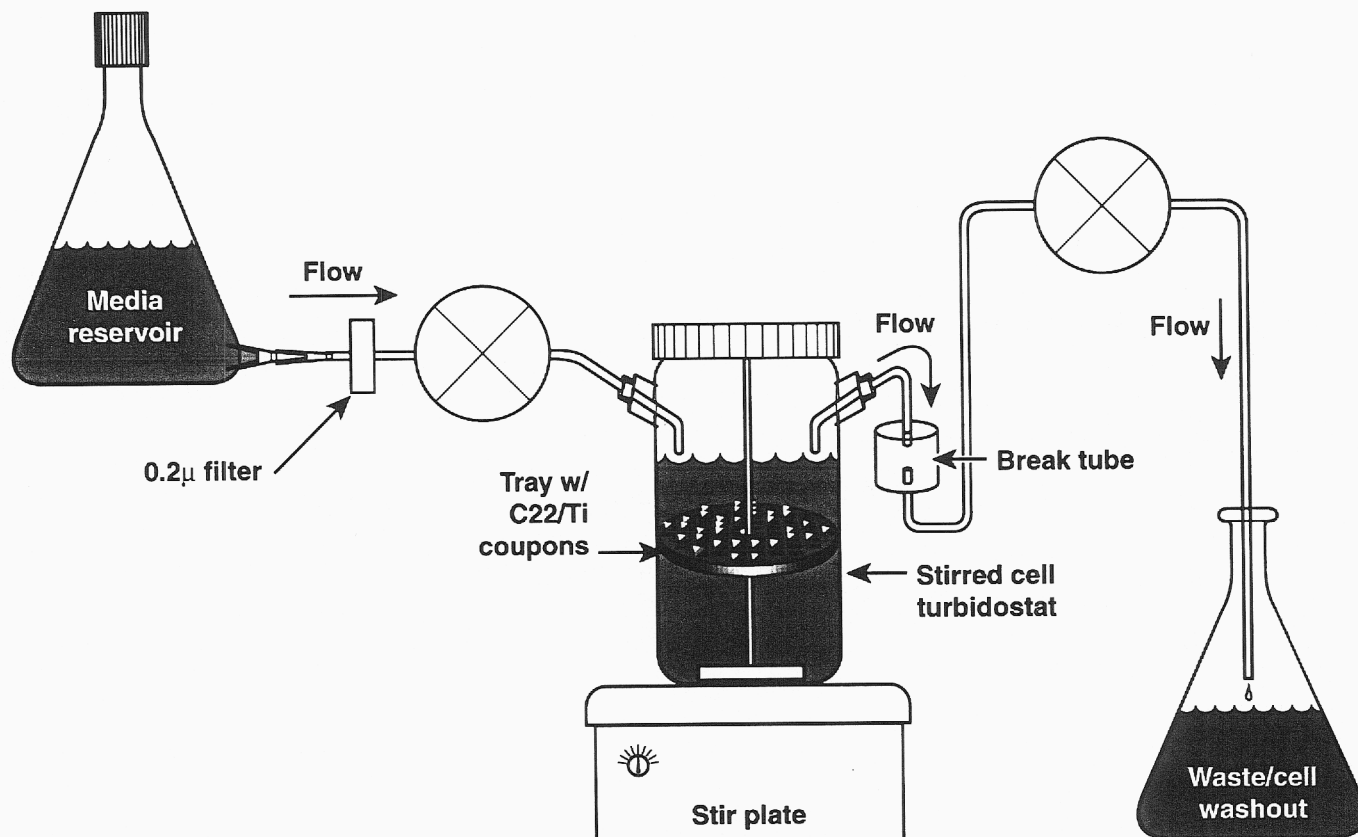


Figure 1. Configuration of the stirred cell *T. ferrooxidans* bioreactor. Sterilized Thiosulfate medium was continuously supplied (3 mL/hr, by means of a peristaltic pump) from a reservoir to a modified 2.5 L stirred cell containing the growing *T. ferrooxidans* culture and material coupons in teflon trays. Media and cells were pumped from the culture (3 mL/hr.) and fluid and cells were effluxed into a waste receptacle. Sterility of the *T. ferrooxidans* culture was maintained by means of an in-line filter on the inlet tubing, and media break tubes on the outlet.

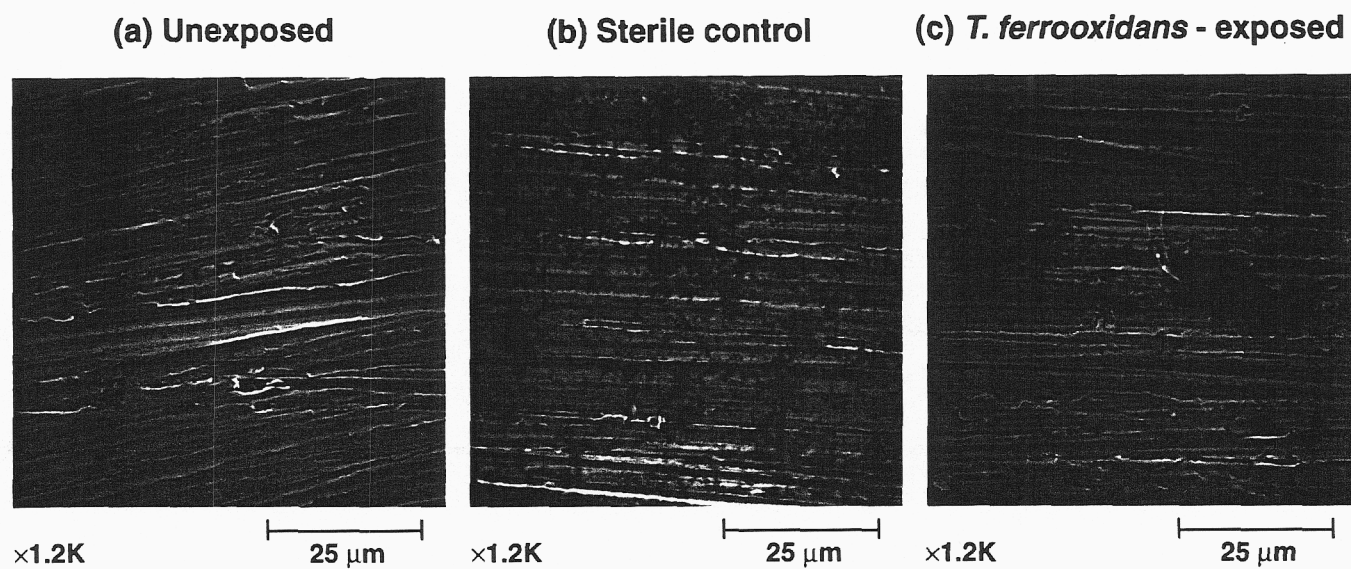


Figure 2. SEM photomicrographs of Alloy 22 after fixation and acid washing. (a), unexposed starting material coupon; (b), after seven months incubation in sterile Thiosulfate medium alone; (c), after seven months incubation in *T. ferrooxidans* culture grown in Thiosulfate medium.

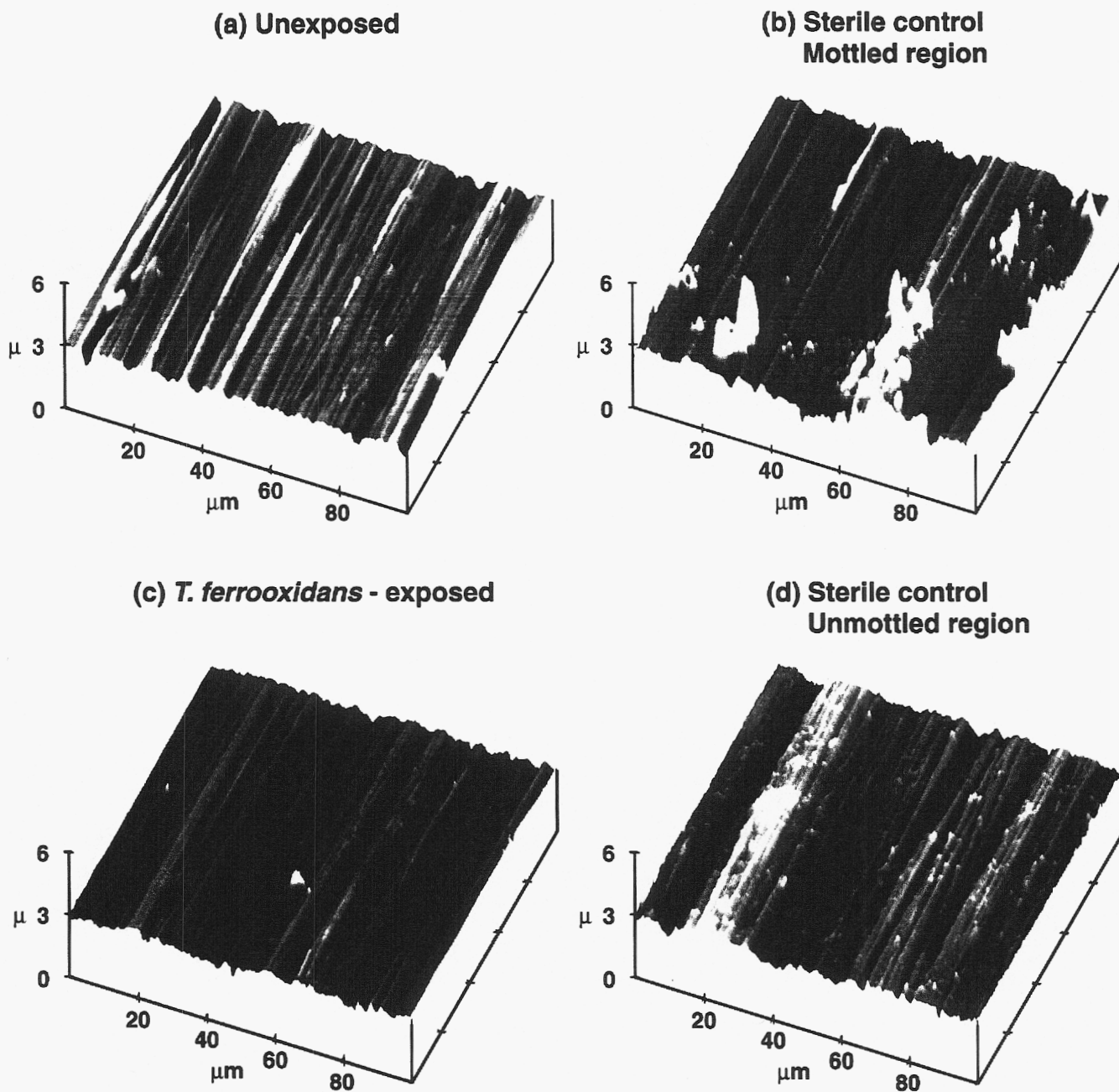


Figure 3. AFM images of Alloy 22 after fixation and acid washing. (a), unexposed starting material coupon; (b), after seven months incubation in sterile Thiosulfate medium alone, region that appeared mottled in SEM photomicrographs; (c), after seven months incubation in *T. ferrooxidans* culture grown in Thiosulfate medium; (d), after seven months incubation in sterile Thiosulfate medium alone, region that appeared unmottled in SEM photomicrographs.

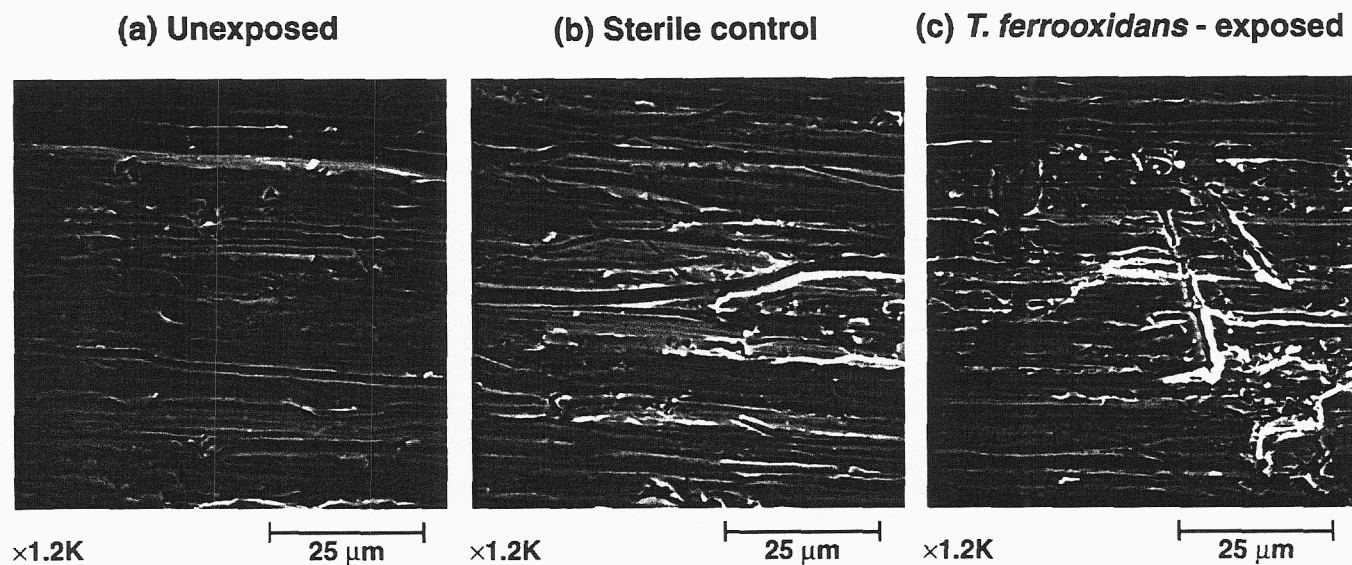


Figure 4. SEM photomicrographs of Titanium grade 7 after fixation and acid washing. (a), unexposed starting material coupon; (b), after seven months incubation in sterile Thiosulfate medium alone; (c), after seven months incubation in *T. ferrooxidans* culture grown in Thiosulfate medium.

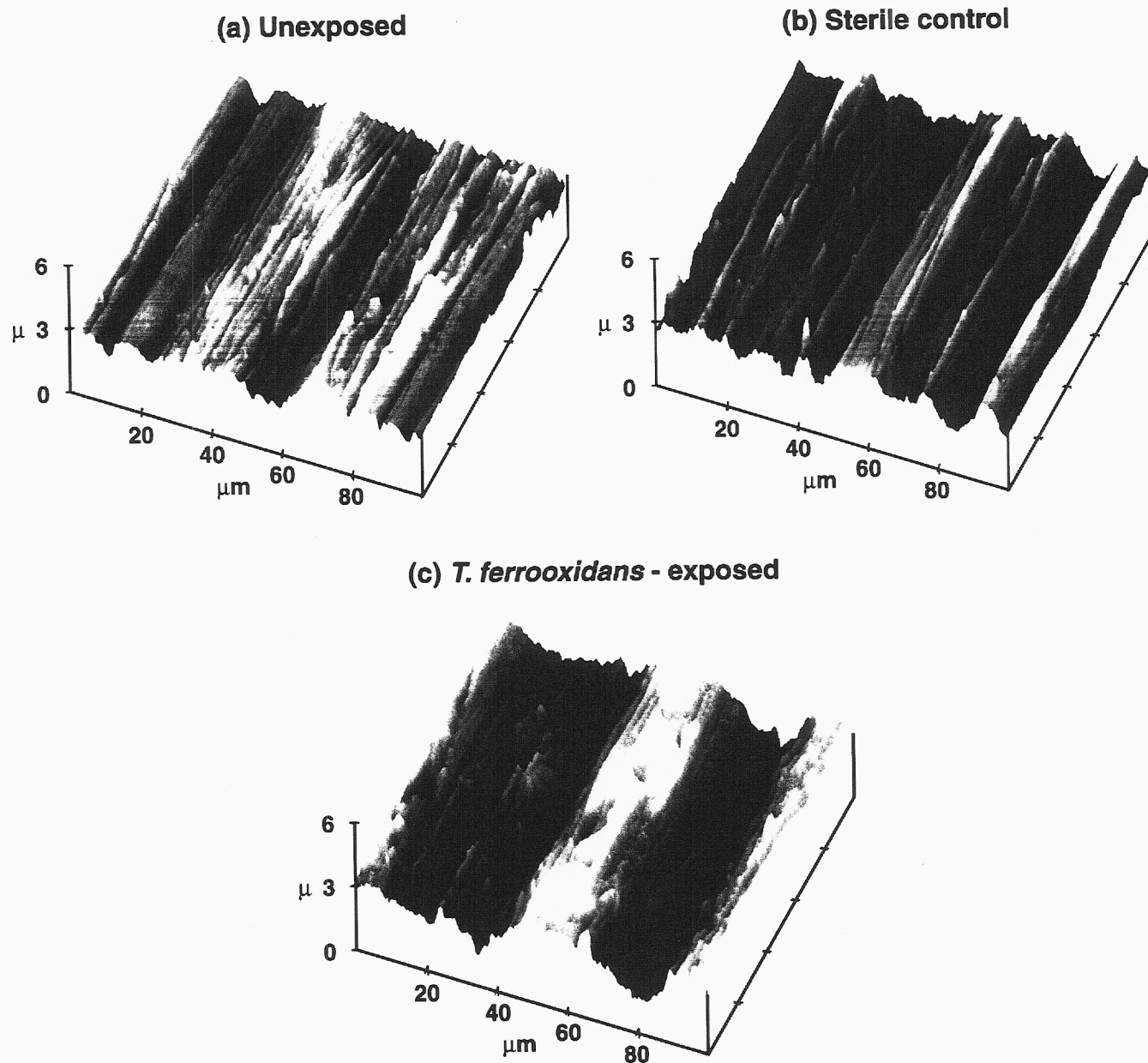


Figure 5. AFM images of Titanium grade 7 after fixation and acid washing. (a), unexposed starting material coupon; (b), after seven months incubation in sterile Thiosulfate medium alone; (c), after seven months incubation in *T. ferrooxidans* culture grown in Thiosulfate medium.

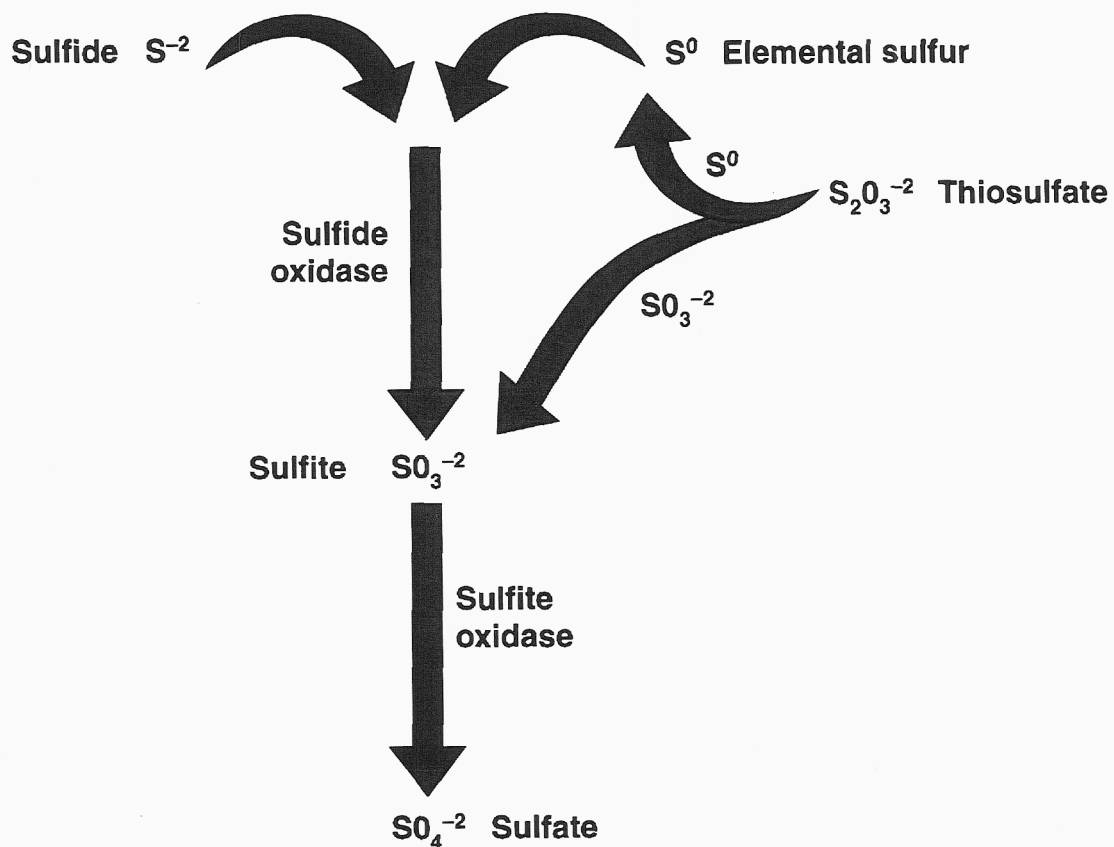


Figure 6. Metabolic pathway of reduced sulfur oxidation by thiobacilli. Thiosulfate is split into sulfite and sulfur, the resulting sulfite is oxidized to sulfate by the enzymatic activity of sulfite oxidase. Elemental sulfur can be oxidized to sulfite by sulfide oxidase if the supply of thiosulfate becomes exhausted. Oxidation of sulfides does not include production of elemental sulfur.